PROPOSAL FOR CONFERENCE

"THE CHEMISTRY AND BIOLOGY OF α_2-MACROGLOBULIN"

The plasma protease inhibitor, alpha-2-macroglobulin, is of interest as a modulator of enzye activity. In addition, results from the studies of this protein bear on three converging lines of research in different fields in biochemistry and cell biology. First, recent studies of the reaction of alpha-2-M with proteases have revealed an unusual mechanism, which may have relevance to other systems involving protein-protein interactions. Also, an unexpected homology has been discovered between alpha-2-M and certain proteins of the complement system. Work on the two protein systems has shown the involvement of a new protein functional group, an internal thiolester. This is the center for reaction of alpha-2-M with other proteins, and of complement with cell surfaces. The discovery of the thiolester has opened a new area of protein chemistry. Finally, alpha-2-M has been a probe for uptake of proteins into cells and the results from chemisal studies have been directly applied to the biological properties of the molecule. This application is a proposal for an international conference to discuss and integrate the recent information on this protein.

I. RATIONALE AND BACKGROUND

Perhaps the best way to appreciate the value of a conference on alpha-2-macroglobulin at this time, is to consider several recent results in this field.

1. In studies of the effect of the reagent methylamine on the alpha-2-macroglobulin molecule, a new chemical group, an internal (cys-glu) thiolester has been discovered in the structure of the protein. This thiolester has been identified as the site for reaction of methylamine (1,2). Evidence has been presented that enzymes bind to this inhibitor at the same site via their lysyl amino groups (3). In other words, amines, which have been known for some time to inhibit alpha-2-M act as an analog of reactive lysyl amino groups of enzymes.

2. This unusual thiolester was actually first observed in proteins of the complement system, where it is the site of interaction with components of cell surfaces to which complement proteins bind. The amino acid sequence containing the thiolester is the same, or closely homologous, in alpha-2-macroglobulin and the C3, C4, and C5 proteins (4,5)

3. The original observation that proteases bound to alpha-2-M have a free active site is, thus, explained by the fact that a covalent attachment at a different site, namely the lysyl amino groups, is effected during binding. The restricted specificity of the bound protease for protein substrates or other inhibitors is due to steric hinderance imposed by the attachment to alpha-2-M. Recent work shows that this restricted specificity is not absolute but rather a kinetic effect, suggesting the possibility that alpha-2-M may have some role as a modulator, as well as an inhibitor, of proteolytic activity.

4. The alpha-2-macroglobulin molecule is taken up by various kinds of cells, and this uptake has become a probe for the study of the general phenomenon of receptor-mediated endocytosis (6). It has been observed that macrophages, or fibroblasts in culture, recognize native alpha-2-macroglobulin poorly, but rather, are specific for the alpha-2-M-enzyme complex or for alpha-2-macroglobulin that has been treated with methylamine (6-8). Likewise, clearance of the inhibitor from plasma is dependent on its having reacted with an amine or protease (9). This result

is consistent with the theory that a biological activity is controlled by a protein isomerization due to reaction at the thiolester binding site. Such an hypothesis relates the results from chemical studies to the physiologic behavior of this inhibitor.

The basic rationale of this proposal is that these results, and others bearing on alpha-2-M biochemistry, have appeared rather suddenly. We are confronted with new facts of basic protein chemistry (internal thiolesters), new mechanisms of protein-protein interactions, and new concepts in the interaction of biomolecules with cells. There are few areas of research where breakthroughs in fundamental biochemistry have so closely paralleled advances in cell biology. Perhaps because of this rapid advance, there has been little opportunity for communication among the various research groups involved in this pioneering work. There has, in fact been no published review encompassing this recent information.

II. OUTLINE OF TOPICS COVERED

Six major areas are envisioned for the conference:

1. BACKGROUND. Recent discoveries in alpha-2-M research touch on important themes in basic chemistry. This section of the conference, in addition to providing a background on the inhibitor itself, will examine two fundamental biochemical questions. First, the chemistry of thiolesters and acyl transfer reactions will be discussed with an eye to the factors that may be involved in the concerted proteolysis and reaction at the thiolester. The definition of the chemical nature of the reactive groups in alpha-2-M by structure-activity relations is already an area of research in some laboratories (10), and this section of the meeting should provide some perspective from the bioorganic chemist's point of view. A second area of fundamental biochemistry to be discussed is the proposed enzyme-inhibitor bond which is chemically identical to the product of transglutaminase reactions and represents a common theme in biology.

2. STRUCTURE. In the past two years, virtually the complete amino acid sequence of alpha-2-macroglobulin has been obtained by Sottrup-Jensen and coworkers -- it is to be expected it will be complete by the projected start of the conference. In addition, the sequences around the active thiolester, proteolytic cleavage sites and other active centers have been determined. Recent results using a variety of physical probes have also appeared and the integration of this structural information is a major aim of the proposed meeting. It is to be emphasized that, in combination with the results on mechanism this should present a rather striking accomplishment for research on a protein that, only a few years ago was considered intractable and somewhat mysterious.

3. MECHANISM The sequence of events, and the number and nature of intermediates will be examined in this section. Particular emphasis will be on the nature of "nascent" alpha-2-macroglobulin, the reactive intermediate that is generated in the course of reaction of the inhibitor with active proteases.

4. COMPLEMENT. The important aspect of recent research in complement, with regard to its relevance to alpha-2-M chemistry is the demonstration of the thiolester bond in complement proteins in an homologous sequence to the one at the reactive site in alpha-2-macroglobulin. The evidence for nucleophilic attack on this center by cell surface components represents a further elaboration on the chemistry of this newly discovered group and is to be seen in light of proposals that endocytosis of the inhibitor may

involve this or some other type of nucleophilic attack on an activated center. It is these aspects of complement that will be discussed in this section of the proposed conference. Again, the direct applicability of chemical principles from two diverse systems to a problem in biology that has wide scope is the major justification for a conference, at this time

5. PHYSIOLOGY AND MEDICINE. This section of the proposed meeting will focus on the interaction of alpha-2-macroglobulin with cells and other macromolecules in a physiologic setting. The role of the inhibitor in diseases will also be a topic of discussion. The question of a role for alpha-2-M in cystic fibrosis is a controversial topic that is projected for this part of the program with major proponents for and against involvement of alpha-2-M in this disease. Implications of results from in vitro studies bearing on emphysema will also be included. Recent evidence suggests that alpha-2-M complexes with elastin may still possess activity against elastin under certain conditions. Proteolytic activity released by macrophages and leukocytes is generally believed to be a causative agent and the unique chemical properties of alpha-2-M enzyme complexes may explain an anomalous breakdown in the control of proteolytic activity.

6. ENDOCYTOSIS. This area of investigation represents one of the most promising for the direct application of the chemical information to the biologic function of alpha-2-M. The major theme here is that endocytosis of the inhibitor is dependent on a conformational change caused by reaction at the thiolester site. That is, the binding of a protease to alpha-2-M brings about a change in the molecule that allows it to be recognized by cells. In addition, the role of a second site (experimentally observed by incorporation of dansyl-cadaverine) will be another area to be explored -- published work suggests it further controls endocytosis of the inhibitor. This system represents an area in which we have a potential molecular handle on macromolecule-cell interactions.

III. SCOPE AND PROJECTED AUDIENCE. Although the specific interest of the conference is a single protein , the areas of research described above make this a topic of broad scope. Thus, the involvement of a new protein group makes it of interest to peptide and protein chemists. Biochemists working in the area of proteolytic enzymes and inhibitors and blood chemists have an obvious interest in this field. The involvement of alpha-2-M in receptor-mediated endocytosis would make the meeting of concern to a broad spectrum of cell biologists. Finally, the potential medical significance described below suggests that the medical community would benefit from such a conference as well.

IV. SIGNIFICANCE. The major function of of alpha-2-macroglobulin in physiology is in regulation of proteolytic activity. It is generally assumed that foreign proteases due to infection or endogenous proteases generated during inflamation or trauma are removed by complexing with the inhibitor which is then cleared from the circulation by endocytosis. Alpha-2-M may also play a role in fibrinolysis. In addition, it has been suggested that this inhibitor is involved in regulation of protease activity in the lung and pancreas, although no definitive studies have been made. The persisting enzyme activity of alpha-2-M protease complexes, however, suggests an obvious lead for investigating those disease states that are characterized by a breakdown in the control of proteolytic activity. As noted above, the elastolytic activity of complexes of alpha-2-M complexes with leukocyte elastase may be an important model of pulmonary emphysema that will be discussed at the proposed conference. There have been several reports of the involvement of alpha-2-M in a number

of disease states and, at least in culture systems, abnormalities are detected in tumor cells. Finally, the possibility that this inhibitor may play a more subtle regulatory role in the generation and maintenance of alpha-2-M-enzyme complexes with restricted specificity is an area of speculation encouraged by its occurence in a wider variety of cells than originally thought, and by the emerging definition of the actual specificity of active inhibitor-enzyme complexes. In general, results from the biochemical investigations have led us to more productive biologic studies. Alpha-2-macroglobulin research appears to be an area where function may emerge from an understanding of structure.

V. REFERENCES.

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CONFERENCE ON THE CHEMISTRY & BIOLOGY OF ALPHA-MACROGLOBULIN

ESTIMATED BUDGET

BUDGET CATEGORY	REQUESTED	EXPECTED FROM OTHER SOURCES (INCLUDING INCOME FROM CONFERENCE)
Personnel		
Salaries prorated		
Administrative		$7,500 - NYAS
Editorial		7,500 - NYAS
Equipment		
Rental of equipment; projection, Poster Boards, Easels	750	
Supplies		
Telephone, telegraph, misc. postage, mailing of programs, etc.	1,000	1,000 - NYAS
Travel		
(1) Domestic Speakers	4,150	
Foreign Speakers	8,604	
(2) Housing and subsistence charges; per diem allowance or actual charges for Speakers ($75/day) number of persons 7 days allowed		
Domestic - 3 days	1,725	
Foreign - 4 days	1,800	
Publication Costs		
(a) Printing of Publication		35,000 - NYAS
(b) Distribution & Mailing		3,000 - NYAS
All Other Expenses		
(1) Arrangements: printing programs & Abstracts & notices	2,500	
Conference Promotion	1,500	
(2) Registration fees (exclusive of dues)		3,500
(3) Rental of conference & accessory space, electrician fees	4,500	
(4) Recording services: recorder/ transcriber	1,500	
(5) Auxiliary Personnel: Receptionists	400	
Projectionist	300	
TOTAL	$28,729	$57,500

The Conference on the Chemistry & Biology of alpha$_2$- macroglobulin is scheduled for January 12-14, 1983 and will be held at the Barbizon-Plaza Hotel in New York City. Approximatel 500 persons are expected to attend. The composition of the audience is expected to be: biochemists in the fields of proteolytic enzymes and inhibitors, blood proteins, hemostasis and coagulation and related areas. Cell biologists in the area of interaction of proteins with cells, particularly receptor-mediated endocytosis will also be interested. The scientific program will be the responsibility of Dr. Richard Feinman, Downstate Medical Center.

The New York Academy of Sciences charges minimal registration fees: Pre-registration - Nonmembers - $70.00; Members - $10.00; Student Nonmembers - $5.00; Student Members - $3.00; In-Person Registration - Nonmembers - $100.00; Members - $40.00; Student Nonmembers - $10.00; Student Members - $5.00 Average registration income is approximately $3,500. This helps to defray some of the Academy's expenses as outlined on the budget.

The Academy Conference Director, Mrs. Ellen A. Marks, will arrange for the publicity of the conference as well as audio-visual aid for the sessions and other administrative services. She will also arrange for recording/transcription of the discussion sessions and will provide subsequent assistance in publishing the proceedings as a volume of the New York Academy of Sciences Annals. The New York Academy of Sciences' Annals is widely distributed both nationally and abroad to over 1,000 institutional libraries and to over 30,000 members of the Academy. Each year the Academy sponsors scientific conferences of high quality in many areas. Their facilities for the necessary organizational support are exceptionally good.

PRINCIPAL INVESTIGATOR PROGRAM DIRECTOR: Richard D. Feinman

BIOGRAPHICAL SKETCH

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Feinman, Richard D.	Associate Professor	July 19, 1940

EDUCATION (Begin with baccalaureate training and include postdoctoral)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
University of Rochester, Rochester, NY	A.B.	1963	Chemistry
University of Oregon, Eugene, OR	Ph.D.	1969	Chemistry

RESEARCH AND/OR PROFESSIONAL EXPERIENCE.

Research Associate	University of Oregon	Inst. of Molecular Biology	1965-1969
Instructor	SUNY Downstate Med. Center	Dept. of Biochemistry	1969-1971
Assistant Professor	"	"	1972-1978
Associate Professor	"	"	1978-present

Recipient: Research Career Development Award HL 00406 1978-1982

Selected Publications

1. Detwiler, T.C. and Feinman, R.D. (1973), Kinetics of Thrombin-Induced Release of Calcium (II) by Platelets. Biochemistry 12, 282

2. Detwiler, T.C. and Feinman, R.D. (1973), Kinetics of Thrombin-Induced Release of Adenosine Triphosphate by Platelets. Comparison with Release of calcium. Biochemistry 12, 2462.

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4. Li, E.H.H., Orton, C. and Feinman, R.D. (1974)., The Interaction of Thrombin and Heparin. Proflavine Dye Binding Studies. Biochemistry 13, 5012.

5. Martin, B., Feinman, R.D. and Detwiler, T.C. (1975), Platelet Secretion Induced by Thrombin and Other Proteases. Biochemistry 14, 1308.

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15. Feinman, R.D. (1979), Kinetics and Mechanism of the Antithrombin-Protease Reaction in The Physiological Inhibitors of Blood Coagulation and Fibrinolysis, Elsevier, 55.

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